Comparative study on the effect of zopiclone and flurazepam

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ABSTRACT

Anxiety disorders are among most frequent mental disorders encountered by Clinicians. Untreated anxiety may result in increase health care utilization, morbidity, mortality and poorer quality of life. Individual with GAD, social phobias and panic disorder show less satisfaction with their quality of life. Basolateral amygdale has been implicated in anxiety generations. Mutations in Sk_3 (calcium channel) are suspected to be underlying cause of severe neurological disorder including anxiety. Therapeutic effects of benzodiazepine are usually or often sustained over months or years, with the need for increased dosage in the treatment of GAD and panic disorders. Flurazepam is a hypnotic agent useful for the treatment of insomnia characterized by frequent nocturnal awakening and other insomnia symptoms such as trouble falling or staying sleep. It acts on BZ_1 and BZ_2 receptor sites. The mechanism of action of zopiclone is similar to benzodiazepines, but it wines more selectively to BZ_1 receptor with similar effects on locomotor activity and on dopamine and serotonin turnover. Zopiclone although molecularly different from benzodiazepines, shares an almost identical pharmacological profile as benzodiazepines including anxiolytic properties. The present study is designed to assess two drugs of different classes that act through same receptor against control for their effects on some of the behavioral activities. Results indicated that zopiclone produced reduction in anxiety more than flurazepam.

Key words: Anxiety, Benzodiazepines, GAD, hypnotic agent, insomnia

1. INTRODUCTION

Anxiety disorders are among most frequent mental disorders encountered by clinicians. Untreated anxiety may result in increase health care utilization, morbidity, mortality and poorer quality of life. Anxiety is of many types, attacks of anxiety that are accompanied by physiological manifestations and people with this disorders often undergoing medical evaluation for symptoms related to heart attacks or other medical conditions. Panic attacks may occur at any time even during sleep.

When the body does not get enough rest required, the brain cannot think clearly, but tries to do it. In this process patient get panic and anxiety attacks. Similarly, when patient suffering from anxiety disorder, they can experience difficulties in sleep patterns, which can lead to insomnia. Insomnia is a symptom, which is characterized by persistent difficulty in falling asleep. It is typically accompanied by functioning impairment when awake. All behavioral and environmental factors that precede sleep may interfere with sleep[1]. Mind body therapy such as stimulus control therapy and cognitive behavioral therapy are particularly helpful. If sleep hygiene do not help, prescription may be an appropriate choice and the best medication is benzodiazepine that include flurazepam. Another class of sedative hypnotic medication includes the non benzodiazepine receptor agonists which include zolpidem, zaleplon and zopiclone.

Benzodiazepines have been found useful in a remarkably wide and varied array of such clinical applications. Most traditional clinical use has been based on their anxiolytic, hypnotic, anticonvulsant and antispastic effects. Other possibly related effects demonstrated in clinical trials and practice includes antipanic, antidepressants, amnestic and anesthetic effects.

Flurazepam is one of benzodiazapines, which has several different effects on body, including relaxing muscles, reducing anxiety, causing sleepiness, stopping seizures and impairing short term memory. Benzodiazepines agonist and other agonist ligands at benzodiazepines site achieve their therapeutic effects by enhancing the action of the inhibitory neurotransmitter GABA (gamma amino butyric acid) at its receptor. The central GABA receptor, known as GABA $_A$ receptor, consist of at least four subunits; three of these alpha, beta and gamma each contain three to six variants. Benzodiazepines binds at the interference of alpha and gamma subunits of $GABA_A$ receptor binding also requires that alpha subunit contains a histidine amino acid residue and hence Benzodiazepine show no affinity for GABA $_A$ receptor containing α 4 and α 6 subunits with arginine instead of histidine[2].

Two GABA receptors have been identified anatomically and pharmacologically. These receptors are variably called type-I and type-II, Benzodiazepine-I and Benzodiazepine -II, or omega-I and omega –II are located out much of the central nervous system. The omega –I site has been associated with the alpha-I subunit, where as the omega –II site appear to be heterogeneous, located on receptors with α-2, α-3 and α-5 subunits. Most of the Benzodiazepines currently available for therapeutic use are consider being full agonists at the Benzodiazepine site. Benzodiazepine also functions as weak adenosine reuptake inhibitors, and the anticonvulsant, anxiolytic and muscle relaxant effects are mediated through $GABA_A$ action[3]. When used to treat anxiety or sleep disorders flurazepam is usually given orally. Flurazepam produces a metabolite with very longer half life (140-250 hours), and is therefore only used for short term

treatment of insomnia, and is considered to be hypnotic[4]. Flurazepam which is mainly used for hypnosis, is oxidized by hepatic enzymes to three active metabolites, Desalkylflurazepam, hydroxymethylflurazepam and flurazepamaldehyde have elimination half-lives ranging 30-100 hours. This may result in unwanted central nervous system depression. In most cases, changes in renal function do not have marked effect on the elimination of parent drugs. The most common side effects of flurazepam in routine clinical use are manifestations of excessive depression of the CNS, adverse effects on other physiological system are rare. Side effects include drowsiness, muscle weakness, light headedness, vertigo, heartburn, nausea, vomiting, ataxia, diplopia, blurring of vision, apathy, severe sedation, disorientation and coma. There have also been rare occurrence of leucopenia, granulocytopenia, sweating, hypotension, shortness of breath, purities, skin rash, dry mouth, euphoria, depression, slurred speech and paradoxical reactions e.g. Excitement, agitation, aggressive behavior and anxiety. The flurazepam with drawl syndrome has been described by many authors. The syndrome can be of considerable severity and have similarities to abstinence syndromes associated with alcohol, opiates and barbiturates. Muscle cramps, vomiting, sweating have occurred following abrupt discontinuation of flurazepam.

BZ_1 receptors that occur in brain areas are involved in sedation, where as BZ_2 are highly concentrated in areas responsible for cognition, memory and psychomotor functioning. While benzodiazepines act selectively on two BZ receptors sub-type, newer such as zopiclone, zolpidem etc are more selective agents and have similar hypnotic activity but reduced CNS unwanted effects. The mechanism of action of zopiclone is similar to benzodiazepines, with similar effects on locomotor activity and on dopamine and serotonin turnover. Zopiclone although molecularly different from benzodiazepines, shares an almost similar pharmacological profile as benzodiazepines including anxiolytic properties. Its mechanism of action is via binding to the benzodiazepine site which in turn positively modulates benzodiazepine sensitive $GABA_A$ receptors to produce zopiclone's pharmacological properties. Zopiclone is the oldest of new compounds considered here, having been introduced in the late 1970s. Most controlled studies of zopiclone have compared the hypnotic effects of 7.5 mg dose with those of benzodiazepines hypnotic dose. In studies using subjective ratings, zopiclone was equivalent to flurazepam 30 mg, triazolam 0.25 mg and nitrazepam 5mg on most or all measures. Zopiclone is used for the treatment of short term insomnia. It is suitable in a dosage from 3.75 to7.5 mg for cases of insomnia that are not accompanied by anxiety. After oral administration, zopiclone is rapidly absorbed, with bioavailability of 80%. The plasma protein binding of zopiclone has been reported to be between 45-80%. It is widely and rapidly distributed to body tissues including the brain and other. It is partly metabolized in the liver in form of inactive N-demethylated derivative and an active N-oxide metabolite. Zopiclone is excreted in urine, saliva and breast milk. The pharmacokinetic of zopiclone is altered by aging and is influenced by renal and hepatic functions[5]. Zopiclone is short to intermediate acting, with half life ranging from 3.5 to 6.5 hours and has no active metabolites. The mechanism of action of zopiclone is same as that of benzodiazepines with similar effects on locomotor activity and on dopamine and serotonin turn over. Zopiclone acts at the benzodiazepine binding site on α 1, 2, 3 & 5 $GABA_A$ containing receptors as full agonists causing an enhancement of the action of GABA to produce the therapeutic and adverse effect of zopiclone. Zopiclone and its active metabolite desmethylzopiclone also inhibit N-methyl-D-aspartate receptor (NMDA) and nicotinic acetylcholine receptors (nACHRs) which might play additive role in addictive properties of these drugs. The most common side effects are taste alteration or dysgeusia (bitter metallic taste). Palpitation may occur at day time following withdrawal from the drug after prolong use. It induces amnesia type memory impairments, impairments to driving skills with a resultant increased risk of road accidents. At the level of CNS, disruption of REM sleep, double vision, drowsiness, headache and fatigue is observed. Abrupt withdrawal particularly with prolonged and high doses can in severe cases causes seizures and delirium[6].

2. PURPOSE OF STUDY

Prescription medications such as benzodiazepines that include flurazepam may cause psychological and physical dependence because of non-selectivity to receptors site. Physical withdrawal symptoms may occur if the drug is tapered for long term use. The newer sedative and hypnotic drugs includes non benzodiazepine, benzodiazepine receptor agonists, zopiclone are more selective for benzodiazepine receptor BZ_1. These newer appear to have better safety profiles and fewer adverse effects than benzodiazepines; they are also associated with low risk of abuse and dependence than benzodiazepines, although dependence occur. The present study is designed to assess two drugs of different classes that act through same receptors against control for their effects on some behavioral activities. The permission for carrying out the research work was granted by department of Pharmacology, University of Karachi.

3. EXPERIMENTAL

The present study was conducted on 30 locally bred Swiss albino rats purchased from HEJ research institute of chemistry. All were male, divided into three groups each comprising of 10 rats, weighing between 150-200gm, housed in standard size poly propylene cages and maintained in reserve light and dark cycle of 12 hours each. Animals were fed on standard diet, water and libitum. Flurazepam HCl and zopiclone were used in the experiment against control animals which were treated with saline only. Dose of the drugs were calculated according to the body weight of animals, zopiclone 7.5 mg/60 kg and flurazepam 15mg/ kg. The tablets of both the drugs were crushed and powdered

and were diluted with water, the suspension was then given to rats with feeding tubes in the morning. The drugs were administered for duration of 4 weeks as follows:

No of animals	Control	Drug Treated
10	Saline	-
10	-	Zopiclone
10	-	Flurazepam

All the animals were weighed before starting the experiment and at the end of the study.
The various experimental parameters monitored during the treatment were as follows:

3.1 *Gross behavior*
All the animals were observed for acute behavioral changes which include: grooming, straub's phenomena, writhing, tremors, twitches, righting reflex, pinna reflex, corneal reflex, exopthalamus, salivation, lacrimation, defecation, urination and motor activity etc.

3.2 *Open field activity*
Animals were kept in the center of the square area measuring 76x76 cms with walls of 72 cm in height and the changes in behavior were observed against control animals exactly after 30 minutes of oral administration, this was continuously repeated on alternate days and observed finally after 28 days.

3.3 *Cage crossing*
The activity of both control and treated animals was observed weekly by placing animals in cage and allow them to move freely and the number of crossings with all four paws was scored for 10 minutes and was observed finally after 28 days.
    Animals were forced to swim in the water tank, as they are not habituated for swimming they tried to escape and struggle for it for 2-3 minutes then stop struggling and start swimming. The duration in which rats struggle, is called struggling time.

3.4 *Exploratory behavior*
To determine this activity each animal was kept in a square box having 16 equally spaced small holes called head dip apparatus. Rats because of inquisitive nature try to explore by dipping head in each hole several times, and tries to escape from these holes. According to the cut off time of 10 minutes, the number of times it dips its head is calculated and noted.

3.5 *Grip Test*
The animals are forced to hang on a wire or rod for a period of ten minutes and note the traction time for which the animals were hanged. The experiment was repeated on alternate days and finally on day 28.

4. STATISTICAL ANALYSIS
Results are presented as mean $\pm$ standard error of the mean (S.E.M). Data on the effects of zopiclone, flurazepam administration as compare to control on cumulative weight gain, gross behavior, open field activity, cage crossing, forced swimming, traction and exploratory activity were statistically analyzed by students "t" test.
Difference between the various groups were considered statistically significant when it is in the acceptance region ($p = 0.05$).

5. RESULTS AND DISCUSSION
Flurazepam binds with BZ_1 and BZ_2 sites and because of its non selectivity, it exerts its action of receptors i.e. psychomotor depression, sedation, cognition and impaired memory, sleep and blurred vision. Zopiclone, a non benzodiazepines, are   more selective for BZ_1 receptors which involves sedation, hypnosis and thus have similar hypnotic activity with less propensity of un wanted CNS adverse effects i.e. drowsiness, fatigue and headache.
    The untreated rats move freely in the previously measured field with mean X = 78.3$\pm$7.8 (Table-1), while the rats treated with zopiclone showed less activity than control with mean 39.8 $\pm$ 5.69 because of sedative and hypnotic effect of the drug. The activity of the animals treated with zopiclone and flurazepam was decreased from control but it was more significant with zopiclone. It has also been reported by Denise and Bocca[7] (2003), that zopiclone reduces motor activity. This indicates that zopiclone produces reduction in motor activity due to its hypnotic action, and due to its action on GABA and NMDA receptors, also zopiclone can produce the effect on nicotinic receptors which might also correlate to its effect on reduction in motor activity observed in open field activity.

Table-1: Effect of drugs on open field activity of animals

Groups	Control	Flurazepam treated	Zopiclone treated
No of models	10	10	10
Mean (X)	78.3 times	46 times	39.8 times
Standard deviation (S.D)	$\pm$ 7.8	$\pm$ 9.79	$\pm$ 5.69

Table-2: Effect of drugs on cage crossing activity of animals

Groups	Control	Flurazepam treated	Zopiclone treated
No of models	10	10	10
Mean (X)	76.8 times	39.2 times	36.2 times
Standard deviation (S.D)	$\pm$ 4.15	$\pm$ 6.33	$\pm$ 8.27

Table-3: Effect of drugs on exploratory activity of animals

Groups	Control	Flurazepam treated	Zopiclone treated
No of models	10	10	10
Mean (X)	52.77 times	27.6 times	27.5 times
Standard deviation (S.D)	$\pm$ 7.3	$\pm$ 4.45	$\pm$ 6.67

Table-4: Effect of drugs on forced swimming activity of animals

Groups	Control	Flurazepam treated	Zopiclone treated
No of models	10	10	10
Mean (X)	1.60 minutes	1.55 minutes	1.32 minutes
Standard deviation (S.D)	$\pm$ 0.37	$\pm$ 0.49	$\pm$ 0.31

The controlled rats showed crossing scored with mean $X = 76.8 \pm 4.15$ (Table-2) while the rats treated with zopiclone showed much decline in scores having mean $X = 36.2 \pm 8.27$ may be due to less energy formation as the drug effect the cyclooxygenase pathway.

The cage crossing was decreased by zopiclone and by flurazepam both as compared to control, however the effects was more significant for zopiclone, this decrease is due to effects on GABA receptor that cause calming action and leads to decrease locomotory activity. This finding is related to its indication and its mechanism of action .It has also been reported by Jeffery .et al.[8] (2001) that zopiclone can affect the locomotory activity.
The rats treated with flurazepam showed less activity than control with mean $X= 39.2 \pm 6.33$, the reduction in the locomotory activity by flurazepam was also significant but the reduction was not as marked as was produce by zopiclone, the reason for this effect is that flurazepam act as partial agonist also and impairment of motor function is not as marked as that for zopiclone. It has also been reported by Ongini .et al.[9] (1981) that flurazepam can cause cGMP lowering; this lowering is not the reason for motor impairment i.e. produced by benzodiazepines. This effect may be due to increased effect of GABA that decrease the model's level of anxiety and may produce effect on BZ_2 receptor that affects the motor activity.

The struggling time shown by the controls were 1.60 ± 0.37 minutes (Table-4) then they start swimming, while the zopiclone treated rats showed the decrease mean struggling time i.e. 1.32 ± 0.31 is observed. This may be due to sedation or CNS depression. The struggling time was decreased because zopiclone has anti anxiety activity and is metabolites have anxiolytic profile. It is because of this effect that the animal has less struggling time, and animal is relaxed and doesn't show any despair to come out of water tank. It is also reported by Bilhard .et al.[10] (1989) that zopiclone can impair hand, eye coordination, which may be a cause also due to which the rats did not show struggling. The effect of flurazepam on swimming induced test was that it decrease the time in few animals but was less than that of zopiclone, this could also be attributed to a fact that zopiclone act as partial agonist of benzodiazepines leading to this effect[11].

The control rats because of inquisitive nature explore by head dipping with mean $X= 52.77 \pm 7.3$(Table-3), but the zopiclone treated rats have decreased exploration counts having mean $X =27.5 \pm 6.67$, which might be due to calming, sedative and calming effect of zopiclone. The activity of zopiclone on exploratory activity was also significant as compare to control and is due to drug anxiolytic profile. This is also reported by Jefferey .et al.[8] (2001), that zopiclone and its metabolite has anxiolytic profile. This leads to calming action, slight sedation and a reduction in exploratory activity. The anxiolytic profile is also observed for flurazepam but due to sedation effect of zopiclone and anxiolytic profile the reduction in exploratory activity is less than that for flurazepam indicating that GABA effects of flurazepam can produce more pronounced effects on the cognition and can significantly the exploratory activity.
The effect of flurazepam on exploratory behaviors of rats is nearly same to that of zopiclone with mean $X=27.6$ that might be due to enhancement of GABA's effects and sleep induction. It is due to fact that flurazeam has anxiolytic activity due to its effect on GABA receptors like other benzodiazepines. This is also in consistent findings of Zhang .et al.[11] (1993), according to which there is a decline in spontaneous activity by flurazepam due to decrease in the rate of neuronal discharge.

All control models shows positive traction and griped the wire, while after treatment with zopiclone most of the rat failed to grip the wire that may be due to sedation and hypnosis induction.

Confusion, drowsiness, sedation, less respond to reflexes and over all decrease in mental activity was observed because of sedative and depressive effect, while urination, defecation and salivation is normal.

The number of gross behavioral effects were observed during the present work includes decrease locomotory activity, zopiclone significantly reduced locomotory activity which is also observed in our study. From the facts that zopiclone inhibits locomotor activity in rats, and inhibits brain dopamine and 5 HT turn over, the mechanism of action of zopiclone in brain neurons is similar to that of benzodiazepine derivatives. Our results of the present research work are in accordance with the research of Liu .et al.[13] (1985).

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